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ART UNIT	**PAPER NUMBER**
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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/685,837	SEIBLER ET AL.	
	Examiner	Art Unit	
	Anoop Singh	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *02 May 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-3,5-24,26,27,29 and 30* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-3,5-24,26,27,29 and 30* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Applicant's amendment filed on May 2, 2006, has been received and entered. Claims 1-6, 9, 12, 13, 14-15, 18, 26-27, 29-30 have been amended and claims 4, 25 and 28 have been canceled.

Claims 1-3, 5-24, 26-27 and 29-30 are pending in the instant application.

Election/Restrictions

Applicant's election with traverse of the invention of group IV (27) filed October 24, 2005 was acknowledged. The traversal was on the grounds(s) that Examiner has not set forth convincing argument that the search and examination of group I and other groups such as II, III, V and VI necessarily represents an undue burden for the examiner and that examination of studying method of knock down in vertebrate, tissue and cell along with elected group directed would not require separate searches for prior art. Applicant argument of examining method for gene knock down in a vertebrate (group 1) with elected group were found persuasive, therefore invention of group I and IV directed to vertebrate and method of gene knock down in a vertebrate are rejoined for the examination purposes. However, Applicant argument for examining other groups (II, III, V, VI) and that examiner must show a burden of searching by separate classification was not persuasive as stated in previous office action.

Applicants in response to office action dated 12/2//2006 argue that independent claim 1 recite nonhuman vertebrate, tissue and cells together and therefore searching all three would not cause an additional search burden. These arguments were not persuasive because examination of different method and/or composition groups would require undue search burden. It is emphasized that examining a method claim for knock down in a cell/tissue is only one limitation and Examiner has to consider the method steps and perform searches. For example, method of introducing a construct to a cell or a tissue would be different from that of a nonhuman vertebrate and thus require

separate searches and search would be an undue burden since method and factors affecting these distinct steps would have to be considered. Additionally, the different inventions have different status in the art because they are drawn to different structure and functions.

The requirement is still deemed proper and is therefore made FINAL.

Claims 1-3, 5-24 and 30(groups II-III and V-VI) drawn to a method for constitutive and/or inducible gene knock down in a tissue culture and cells have been withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on October 24, 2005.

Accordingly, a method for gene knock down in a vertebrate and vertebrate having stable integration at Polymerase II dependent locus, an expression vector comprising an shRNA construct under control of a ubiquitous promoter will be examined in the instant application.

Claims 1-3, 5-24, 26-27 and 29-30 are under consideration.

It is noted that the claims 1-3, 5-24, 26-27 and 29-30 of groups I and IV are included in multiple groups because they encompass the inventions of these groups. However these claims will be examined to the extent they encompass the invention of the elected group, a nonhuman vertebrate and method of gene knockdown in a nonhuman vertebrate having stably integrated, at a polymerase II dependent locus of the vertebrate, an expression vector comprising a short hairpin RNA (shRNA) construct under control of a ubiquitous promoter.

Maintained-Claim Objections

Claims 1-3, 5-24, 26-27 and 29-30 are objected to because of the following informalities: Claims 1-3, 5-24, 26-27 and 29-30 continue to depend in part to claims that are withdrawn and should be rewritten in independent form to recite elected invention. It is noted that an election was made to prosecute vertebrate and a method

for gene knockdown in a vertebrate as discussed above. Claims still encompass "tissue culture" and "cells" which are not part of the elected invention. Appropriate correction is required.

New-Claim Objections

Claim 2 is objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. In the instant case, claim 2 does not further limit the method of claim 1 since the limitation of stable integration of expression vector in the genome of the nonhuman vertebrate is already recited in claim 1 and thus, claim 2 does not further limit claim 1. Appropriate correction is required.

Withdrawn-Claim Rejections - 35 USC § 101

Applicant's amendments in claims 1-6, 9, 12, 13, 14-15, 18, 26-27 are found persuasive. Therefore, claims previously rejected under 35 U.S.C. 101, as directed to non-statutory subject matter are withdrawn.

Withdrawn-Claim Rejections - 35 USC § 112

Applicant's amendment in claims 2-4, 6, 9, 12, 14-15, 18 and 27 are found persuasive. Therefore, claims 2-4, 6, 9, 12, 14-15, 18 and 27 previously rejected under 35 USC § 112 as being vague and indefinite are withdrawn.

New Claim Rejection- 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 29 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The instant invention claims the nonhuman vertebrate of claim 27, which is or is derived from mouse or fish. It is uclear how one can derive any nonhuman vertebrate from mouse or fish. It is emphasized that "derive" can have a broad range of interpretations. For instance, **Derive:** To obtain or receive from a source.

1. To arrive at by reasoning; deduce or infer: *derive a conclusion from facts.*
2. To trace the origin or development of (a word).
3. *Linguistics.* To generate (a surface structure) from a deep structure.
4. *Chemistry.* To produce or obtain (a compound) from another substance by chemical reaction.

http://dictionary.reference.com/search?q=derived%3E

Given the breadth of the definition of "derived", the metes and bounds of the nonhuman vertebrate "derived" from mouse or fish are unclear. Further, it appears to omit essential steps if claims intend to "derive" other species of vertebrate from nonhuman vertebrate.

New grounds of Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-3, 5-24, 26-27 and 29 remain rejected under 35 U.S.C. 112, first paragraph, and newly added claim 29 is also rejected because the specification, while being

enabling for a method of gene knockdown in a mouse genome at the *rosa26* locus, said method comprising introducing a shRNA and reporter constructs in mouse embryonic stem cell by homologous recombination, wherein said shRNA and reporter constructs comprises a gene encoding Renilla (Rluc) and luciferase (Fluc) along with an adenovirus splice acceptor sequence and polyadenlylation signal placed downstream of the endogenous promoter of rosa26, and Fluc specific shRNA expressed under the control of H1 and U6 promoter and terminated by five thymidines; and microinjecting said mouse embryonic stem cell into mouse diploid blastocysts; and implanting the blastocysts comprising the mouse embryonic stem cell into pseudo pregnant mouse; allowing the resulting pregnant mouse to deliver viable chimeric offspring and a transgenic mouse produced by said method, wherein said transgenic mouse exhibits ~90% reduced luciferase activity in liver, heart, brain and muscle, does not reasonably provide enablement for the method of gene knockdown in any other nonhuman vertebrate using any other promoter with any other shRNA sequence. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Claim 1 encompasses a method for constitutive and/or inducible gene knock down in a nonhuman vertebrate comprising an shRNA construct under control of a ubiquitous promoter into the polymerase II dependent locus of the genome of the nonhuman vertebrate. The dependent claims 2-3 and 5 limit the expression vector being suitable for stable integration in genome and contains homologous sequence for integration at defined locus through homologous recombination at polymerase II dependent locus selected from list of a group consisting of rosa26, collagen, RNA polymerase, actin and HPRT locus. Claims 6-14 encompass the expression vector of claim 1, which further contains functional sequences and group of promoters, which is either constitutive or inducible. The inducible promoter expression is a promoter consisting an operator sequence and the vertebrate of claim 1 is non-human vertebrate, which is further limited to either mouse or fish. Claims 15-19 encompass method of claim 1 wherein the vector is a either Pol III or Pol II dependent promoter driven shRNA

construct suitable for integration into a Pol II dependent locus. Subsequent claim limit the Pol III promoter being either constitutive or inducible H1 or U6 promoter while Pol II promoter being inducible CMV promoter.Claims 20-27 encompass method of claim 1 which describes the shRNA segment comprising a stop and or polyadenylation sequence which is integrated at polymerase dependent locus of the vertebrate and ES cell of the vertebrate.

The application as filed is not enabling for the invention commensurate with the full scope of the claims because art of gene knockdown by shRNA using any method and any vector is unpredictable as has been recognized by the art of skill and therefore require undue experimentation. As will be shown below, these broad aspects as well as limitations were not enabled for the claimed invention commensurate with the full scope of the claims at the time of filling of this application because neither the specification nor the art of record taught sufficient guidance to practice the claimed invention commensurate with the scope of the claim.

In determining whether Applicant's claims are enabled, it must be found that one of skill in the art at the time of invention by applicant would not have had to perform "undue experimentation" to make and/or use the invention claimed. Such a determination is not a simple factual consideration, but is a conclusion reached by weighing at least eight factors as set forth in In re Wands, 858 F.2d at 737, 8 USPQ 1400, 2d at 1404. Such factors are: (1) The breadth of the claims; (2) The nature of the invention; (3) The state of the art; (4) The level of one of ordinary skill in the art; (5) The level of predictability in the art; (6) The amount of direction and guidance provided by Applicant; (7) The existence of working examples; and (8) The quantity of experimentation needed to make and/or use the invention.

These factors will be analyzed, in turn, to demonstrate that one of ordinary skill in the art would have had to perform "undue experimentation" to make and/or use the invention and therefore, applicant's claims are not enabled commensurate with the full scope of the claims.

Furthermore, USPTO does not have laboratory facilities to test if an invention will function as claimed when working example are not disclosed in the specification,

therefore enablement issues are raised and discussed based on the state of knowledge pertinent to an art at the time of the invention, therefore, skepticism raised in enablement rejections are those raised in the art by artisan of expertise.

Claims 1-3, 5-24, 26-27 and 29 are broad in scope. The following paragraph will outline the full scope of the claims:

Claimed invention recites a method of gene knockdown in a nonhuman vertebrate, wherein said animal comprises stably integrated, at polymerase II dependent locus of the vertebrate, an expression vector comprising a short hairpin RNA (shRNA) constructs under control of any ubiquitous promoter. Since these claims are broad in scope, encompassing any nonhuman vertebrate having stably integrated at Pol II dependent locus a construct, subsequently limiting to few construct under control of any ubiquitous promoter, the disclosure provided by the applicant, in view of prior art, must encompass a wide area of knowledge to a reasonably comprehensive extent. In other word each of those, aspect considered broad must be shown to a reasonable extent so that one of the ordinary skills in the art at the time of invention by applicant would be able to practice the invention without any undue burden being on such Artisan.

The specification broadly discloses the progression of RNA interference technology over the years (pp. 1,2) and describes role of shRNA-mediated gene silencing in transgenic mice and rats. The invention is based in part of a method of using a expression vector comprising a short hairpin RNA construct under the control of ubiquitous promoter for gene knock down in a living organism (pp 3). Pages 4-6 provide short description of figures. Pages 7-14 of the specification disclose definition of terms, general description of ubiquitous promoter, expression vector and a general description of different shRNA sequence in tabular form. Pages 15-17 broadly discusses preferred embodiments of the method steps comprising of generating shRNA and construct for cell culture, luciferase measurement assay and generation of chimeric mice.

Example 1: of specification teaches the firefly luciferase gene along with a splice acceptor sequence is inserted into first allele of rosa26 locus by homologous recombination in ES cells while shRNA and Renilla luciferase gene is inserted into second allele of rosa 26. Figure 7 shows the expression of the firefly luciferase in

presence and absence of shRNA expression cassette. Example 2 shows shRNA expression cassette under control of U6 promoter containing tet operator sequence and a Renilla luciferase gene is inserted into first allele of rosa26 locus (figure 8 and 12), while the luciferase gene with a promoter and a tet repressor expression cassette is introduced into the second allele in ES cells. Luciferece activity is shown in presence and absence of doxycycline. Example 3 page 19 describes that NIH3T3 cells are transiently transfected with construct expressing the luciferase and tet repressor gene together with the shRNA construct containing tet operator sequence. Figure 11 shows the expression of luciferase in presence and absence of doxycycline. The specification discloses doxycycline inducible shRNA expression resulted ~80% inhibition in firefly luciferase activity in cells. Example 4 pages 19 show chimeric mice from rosa26/U6 and H1-ShRNA transgene. The data shows shRNA construct under the control of both U6 and H1 effectively repressed the firefly luciferase activity inmost organs (Figure 13B).

However, such broad disclosure does not demonstrate the information required by the Artisan to reasonably make and use any nonhuman vertebrate with an expression vector comprising any short hairpin RNA (shRNA) construct under control of a any ubiquitous promoter integrated at Polymerase II locus by any method.

As a first issue, claim 1 embraces a method for gene knockdown in a nonhuman vertebrate comprising stably integrated an expression vector comprising a shRNA construct under control of any ubiquitous promoter into a polymerase II dependent locus of the genome of the non human vertebrate. The specification contemplated that the expression vector of the instant invention is suitable for stable integration into the nonhuman vertebrate. It is noted that vectors for transient integration and vector that contains homologous sequences suitable for targeted integration at a polymerase II dependent locus of the nonhuman vertebrate are also contemplated (see paragraph 32). The specification provides working example showing a method of gene knockdown by introducing a shRNA and reporter constructs in mouse embryonic stem cell by homologous recombination, wherein said shRNA and reporter constructs comprises a gene encoding Renilla (Rluc) and luciferase (Fluc) along with an adenovirus splice

acceptor sequence and polyadenlylation signal placed downstream of the endogenous promoter of rosa26, and Fluc specific shRNA expressed under the control of H1 and U6 promoter (see example 1 and 4).

As recited claims 1, 26 and 27 are broad and embrace integration of shRNA construct at polymerase II dependent locus of the genome of the animal by any method. Prior to instant invention, shRNA mediated RNAi in nonhuman vertebrate especially in mice had been shown by a number of independent group using random transgnesis by pronuclear injection and transfection of shRNA construct. It is noted that each of these method resulted in aberrant pattern of shRNA expression depending on the site of transgene integration. In addition, gene silencing in these animals varied from undetectable to greater than 90% level (Tiscornia G, Proc Natl Acad Sci U S A. 2003; 100(4): 1844-8; Hasuwa et al FEBS Lett. 2002; 532(1-2): 227-30, IDS). Furthermore, these methods also failed to show any distinct phenotype (Carmell et al., Nat Struct Biol. 2003; 10(2): 91-92). In the instant case, Applicant's examples only describe a method to knock down expression of luciferase using shRNA construct using mouse ES cell and locus specific targeting of the construct. It is noted that none of the examples demonstrate specific *in vivo* gene silencing by any other method that correlate to stable integration of shRNA construct at polymerase II dependent locus leading to effective gene silencing. It is evident from the cited arts that without any specific guidance of how any method that integrates randomly at polymerase II dependent locus would result in sustained reproducible expression of shRNA resulting in gene silencing. Because of the art, as shown above, does not disclose how even random stable integration of shRNA construct to any locus in the genome would not result in effective gene silencing. An artisan would have to carry out extensive experimentation to make use the invention, and such experimentation would have been undue because of the art of gene silencing using shRNA construct *in vivo* is unpredictable and specification fails to provide any guidance as to how the claimed method would have been practiced.

As a second issue, Claims 1, 3 26-27 and 29 embrace a method that is directed to a nonhuman vertebrate and a method for constitutive gene knockdown in a nonhuman vertebrate whose genome is modified and may requires embryonic stem cell

for stable, locus dependent integration of the construct. The art at the time of filing further held that transgenic technology was not predictable for any species other than mouse. Since the specification discloses using mouse ES cells to produce transgenic mice via homologous recombination of targeting vectors in the ES cells, ES cells from various species are required to produce various vertebrates. However, Houdebine, 1994 (Journal of Biotechnology, Vol., 34, pp 269-287) describes that although ES cells can be used to generate transgenic animals, but this approach remains restricted to mice, ES cells from other species are not presently available (pp 279). Furthermore Mullin et al also point that non-mouse ES cell capable of providing germ line chimeras were not available (Mullins et al., Journal of Clinical Investigation, 1996, pp 1557, 1st paragraph). Campbell and Wilmut (1997, Therigenology) acknowledges report of ES-like cells in number of species, but also emphasize that there are no report of any cell line that contribute to germ line in any species other than mouse (pp 65; 2nd paragraph). Thus, the state of the art is such that ES cell technology is generally limited to the mouse system and that only putative ES cells exist for other species (Moreadith et al., J. Mol. Med., 1997 p214, abstract). Therefore, at the time of filing of this application, method of gene knockdown in any vertebrate could not be accomplished for any species other than mouse. The specification does not teach how to make knock down vertebrate by shRNA for any other species other than mice or correlate making mice to making knockout for any other species. Therefore, the claims should be limited to mouse and method for gene knockdown in mouse as discussed in the office action.

As a third issue, claims 27 and 29 recites a nonhuman vertebrate with an expression vector comprising a short hairpin RNA (shRNA) construct under control of a ubiquitous promoter integrated at a polymerase II dependent locus. However, the specification as filed does not provide any specific information about resulting phenotype of the claimed invention. It is noted that the specification merely recites the luciferase activity in different organ, however it dose not provide any specific information for practicing the claimed invention commensurate with the full scope of the claim. The specification teaches method of making a transgenic mice-using shRNA. However, it is noted that art recognizes that the resultant phenotype, when producing knockdown

mice, is exceedingly unpredictable. Leonard (Immunological Rev., 1995, 148: 98-114) discloses mice with disruption in the gc gene that was intended to be a model for X-linked severe combined immunodeficiency (XCIDS), but displays a variety of unexpected traits (Abstract). These knockout mice were expected to have thymocytes with decreased proliferation in response to stimulation with antibodies, but the thymocytes proliferated normally (pp 105, line 7). Similarly, Carmell et al failed to produce any distinct phenotype, while shRNA, constructs directed against seven known targets were introduced via standard trasngenesis (see pp 91, col. 2, para. 2; Carmell Nat Struct Biol. 2003; 91-92). Thus, it is clear from the cited arts that the resulting phenotype of a gene knockdown resulting from methods routinely used for integrating shRNA in the genome of nonhuman vertebrate was considered unpredictable.

As a final issue, claims are directed to shRNA under the control of ubiquitous promoter and further describes expression vector is also suitable for transient integration (pp7; 4th paragraph). The unpredictability of attenuating /inhibiting expression of a target gene in cell by shRNA is evident in prior and post filing art. While it is recognized, that introduction of shRNA that is targeted to a specific gene may result in attenuation /inhibition of the targeted gene, the degree of attenuation and length of the time attenuation is achieved in not predictable (*supra*). In addition, Prawitt describes a recent study showing expression of shRNA in mammalian cell induced target gene for interferon pathways (Prawitt et al Cytogenet Genome Res. 105 (2-4), 412-421, 2004 pp419, column 1, 2nd paragraph, references therein). In view of these studies, Prawitt et al stressed the importance of interpreting the RNAi effects both in tissue culture as well as in mouse. Furthermore, Prawitt et al evinces an optimistic outlook for tetracycline inducible system in generating inducible knock down mouse, but also acknowledges that the art is still unpredictable by stating "it remains to be proven if doxycycline can be used to study as graded knock down phenotype in mouse" (pp 419; 2nd column).

In view of lack of teaching or guidance provided by the specification with regard to an enabled method for gene knockdown in any vertebrate comprising a disruption in gene using any shRNA, construct comprising different constitutive or inducible

promoter, locus and shRNA sequence and the lack of teaching or guidance provided by the specification to overcome the art recognized unpredictability of disruption of a particular gene, promoter and locus and the resulting phenotype and absence of any correlation between disruption and its phenotype, for the specific reason cited above in the office action. It would require undue experimentation for an Artisan to make and use the claimed invention and/or working examples demonstrating the same, such invention as claimed by the applicant is not enabled for the claimed inventions commensurate with the full scope of the claims.

Withdrawn-Claim Rejections - 35 USC § 112-First Paragraph

Applicant's amendment in claims 1-3, and 26-27 are found persuasive. Therefore, claims 1-27 previously rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement are withdrawn.

Withdrawn-Claim Rejections - 35 USC § 102

Applicant's arguments and amendments, filed May 8, 2006, with respect to the rejection(s) of claim(s) 30 under 102(b) by Paddison and claims 1-2, 5, 7-9, 13-14, 26-27 and 30 by McCaffrey et al, have been fully considered and are persuasive. Therefore, the rejection has been withdrawn. However, upon further consideration, a new ground(s) of rejection is made in view of amendments in claims.

New-Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

(e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

Claims 27-, 29-30 are rejected under 35 U.S.C. 102(b) as being anticipated by Buvoli et al (Mol Cell Biol. 2000; 20(9): 3116-24).

Claims are directed to a nonhuman vertebrate having stably integrated at a polymerase II dependent locus of the nonhuman vertebrate and expression vector comprising a short hairpin RNA construct under control of a ubiquitous promoter. Subsequent claim limits the nonhuman animal to include mouse or fish. Claim 30 is directed to an expression vector comprising an shRNA construct under the control of ubiquitous promoter and a homologous sequence at a polymerase II dependent locus.

Buvoli et al teach a construct ptRNA 8mer/105 and $tRNA^{ser}$ Su+ orche gene multimerization by re-cloning the tRNAsu+ gene into plasmid pUC18 (see material methods page 3116 and figure 1). Since, claim 30 does not provide any specific structure of the claimed shRNA or promoter; thus, an endogenous promoter would meet the claim limitation. Buvoli et al also disclose that efficient tRNA expression and processing could occur with two adjacent tRNAsu+ genes that are separated by 105nt. It is further noted that transcription of human serine tRNA is positively controlled by flanking promoter elements located between –66 and –18 (see page 3122, column 2, paragraph 5). Furthermore, Buvoli et al also teach testing tRNA suppressor gene construct *in vivo* by directly injecting into mouse muscle (see page 3121, column 1paragraph 3). Since claim 27 require a nonhuman vertebrate comprising an shRNA construct under the control of ubiquitous promoters, without indicating what is integrated reads on endogenous tRNA. Therefore, instant claims would read on endogenous tRNA.

Accordingly, Buvoli et al anticipate claims 27, 29 and 30.

Claims 1-2, 5, 7-11, 13-14, 24, 26-27 and 29 are rejected under 35 U.S.C. 102(e) as being anticipated by Beach et al. (US patent Publication no. 2003/0084471, dated 5/1/2003, effective filing date 1/22/2002).

The changes made to 35 U.S.C. 102(e) by the American Inventors Protection Act of 1999 (AIPA) and the Intellectual Property and High Technology Technical Amendments Act of 2002 do not apply when the reference is a U.S. patent resulting directly or indirectly from an international application filed before November 29, 2000. Therefore, the prior art date of the reference is determined under 35 U.S.C. 102(e) prior to the amendment by the AIPA (pre-AIPA 35 U.S.C. 102(e)).

Beach et al disclose that the double-stranded structure may be formed by a single self-complementary RNA strand or two complementary RNA strands. RNA duplex formation may be initiated either inside or outside the cell. Inhibition is sequence-specific in that nucleotide sequences corresponding to the duplex region of the RNA are targeted for genetic inhibition (pp4, paragraph 52). Beach et al teach the length of the dsRNA is at least 20, 21 or 22 nucleotides in length, e.g., corresponding in size to RNA products produced by Dicer-dependent cleavage. In certain embodiments, the dsRNA construct is at least 25, 50, 100, 200, 300 or 400 bases (pp13, paragraph 16). Beach et al disclose that the dsRNA construct may be synthesized either *in vivo* or *in vitro*. RNA can be derived from an expression construct (pp 13,14; paragraph 168). The invention also discloses strategy for stable expression of dsRNA in cultured mammalian cells (Figure 27, paragraph 78). Beach et al disclose generating several types of short dsRNAs corresponding to the coding region of firefly or Renilla luciferase (pp22; paragraph 246). Beach et al demonstrates that short hairpins encoded on a plasmid are effective in suppressing luciferase gene expression (Figure 42) *in vivo*. DNA oligonucleotides encoding 29 nucleotide hairpins corresponding to firefly luciferase were inserted into a vector containing the U6 promoter. Beach further disclose that one of skill can choose from amongst a range of vectors to either transiently or stably express a short hairpin. Beach et al also disclose non-limiting examples of vectors and

strategies to stably express short dsRNAs using U6 and H1 promoters (pp23; paragraph 252; Figures. 43-45). In addition, Beach et al teach expression of the targeted gene *in vivo* also include the cytomegalovirus (CMV) promoter/enhancer (paragraph 147). Beach et al teach and claim a non-human transgenic mammal having germline and/or somatic cells comprising a transgene encoding a dsRNA construct (pp 26, claim 28 and pp 2 paragraph 52) that includes rodent (pp12, paragraph 154). Beach et al also demonstrates that a short hairpin is highly effective in specifically suppressing gene expression of firefly or Renilla luciferase (Example 6). Since, Beach taught a method for luciferase gene suppression using a construct under the control of a ubiquitous promoter, it is inherent that it would randomly integrate into polymerase II dependent locus in order to induce luciferase gene suppression.

Accordingly, Beach et al anticipate claims 1-2, 5, 7-11, 13-14, 24, 26-27and 29.

Response to Arguments

Applicant's arguments filed May 2, 2006 have been fully considered but they are not persuasive. Applicants point out that the rejected claims all incorporate the substance of previous claim 4, which is not subject to rejection. In response, as stated in this office action, subject matter of claim 4 is also anticipated by Beach as it only required construct of invention to be integrate at polymerase II dependent locus. Beach et al teach suppression of luciferase gene *in vivo* and contemplated vectors that inherently would randomly integrate at the polymerase II dependent locus. Furthermore, contrary to applicants argument Beach et al teach a transgenic mouse comprising an expression vector containing shRNA. It is emphasized that Beach contemplated mouse germline cells comprising a transgene encoding a construct and taught strategies for stably expressing an short hairpin (*supra*). It is also made of record that this claim was inadvertently omitted in previous rejection under USC 102 (e) in office action dated 12/02/05. Applicant also assert that beach does not teach a person skilled in the art to how these shRNA construct can be integrated into the genome. Applicant's arguments have been fully considered but they are not persuasive. In response to applicant's

argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., how these shRNA construct would integrate) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993). The rejected claims describe only one method step that is stably integrating expression vector comprising shRNA in to the genome of mice. Since, steps recited in the instant invention are the same as those taught by Beach and do not require how construct are integrated.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 1-3, 5-10, 13-24, 26-27 and 29-30 rejected under 35 U.S.C. 103(a) as being unpatentable over Beach et al. (US patent Publication no. 2003/0084471, dated 5/1/2003, effective filing date 1/22/2002); Bronson et al (Proc Natl Acad Sci U S A 1996; 93:9067–9072) and Soriano et al (US patent 6,461,864, October 8, 2002).

Beach et al disclose that the double-stranded structure may be formed by a single self-complementary RNA strand or two complementary RNA strands. RNA duplex formation may be initiated either inside or outside the cell. Inhibition is sequence-

specific in that nucleotide sequences corresponding to the duplex region of the RNA are targeted for genetic inhibition (pp4, paragraph 52). Beach et al teach the length of the dsRNA is at least 20, 21 or 22 nucleotides in length, e.g., corresponding in size to RNA products produced by Dicer-dependent cleavage. In certain embodiments, the dsRNA construct is at least 25, 50, 100, 200, 300 or 400 bases (pp13, paragraph 16). Beach et al disclose that the dsRNA construct may be synthesized either *in vivo* or *in vitro*. RNA can be derived from an expression construct (pp 13,14; paragraph 168). The invention also discloses strategy for stable expression of dsRNA in cultured mammalian cells (Figure 27, paragraph 78). Beach et al disclose generating several types of short dsRNAs corresponding to the coding region of firefly or Renilla luciferase (pp22; paragraph 246). Beach et al demonstrates that short hairpins encoded on a plasmid are effective in suppressing luciferase gene expression (Figure 42) *in vivo*. DNA oligonucleotides encoding 29 nucleotide hairpins corresponding to firefly luciferase were inserted into a vector containing the U6 promoter. Beach further disclose that one of skill can choose from amongst a range of vectors to either transiently or stably express a short hairpin. Beach et al also disclose non-limiting examples of vectors and strategies to stably express short dsRNAs using U6 and H1 promoters (pp23; paragraph 252; Figures. 43-45). Beach et al teach and claim a non-human transgenic mammal having germline and/or somatic cells comprising a transgene encoding a dsRNA construct (pp 26, claim 28 and pp 2 paragraph 52) that includes rodent (pp12, paragraph 154). Beach et al also demonstrates that a short hairpin is highly effective in specifically suppressing gene expression of firefly or Renilla luciferase (Example 6). Since, Beach taught a method for luciferase gene suppression using a construct under the control of a ubiquitous promoter, it is inherent that it would randomly integrate into polymerase II dependent locus in order to induce luciferase gene suppression. However, Beach et al do not explicitly teach how an expression vector integrates through homologous recombination at polymerase II dependent locus.

Prior to instant invention, Bronson describes transgenic mice made by pro nuclear injection of DNA as an effective method of achieving expression of exogenous DNA sequences for many purposes, including over expression, mutant analysis,

promoter analysis (see page 9067, column 1, para 1). Bronson also describes problems associated with DNA incorporated into the mouse germ line using this method includes random integration and unpredictable copy numbers. It is noted that Bronson provided motivation of targeting a single copy of a transgenic sequence to a chosen location in the genome such as HPRT. He discloses many advantages of targeting at specific locus including the ability to control copy number, the ability to insert the transgene into regions of chromatin compatible with a desired developmental and tissue-specific expression. Bronson also taught homologous recombination in murine ES cells to generate mice having a single-copy of a transgene inserted at a chosen site in the genome (see page figure 2 and page 9068, column 2, para 3). However, Bronson et al do not teach expressing shRNA in a specific locus.

Soriano et al teach methods and vector constructs for the production of genetically engineered non-human animals, which ubiquitously express a heterologous DNA segment in Rosa 26 locus (abstract and claim 1). It is noted that Soriano describes targeting region as a portion of a targeting construct which becomes integrated into an endogenous chromosomal location following homologous recombination between a homology clamp and an endogenous gene locus, such as a ROSA26, ROSA5, ROSA23, ROSA11, G3BP (BT5), or EphA2 gene locus sequence (column 3, lines 51-54. Soriano also discloses a schematic of G2BP gene showing the retroviral promoter trap insertion site and a cassette comprising the ROSAbgeo retroviral insert. Soriano also shows structural motifs associated with RNA binding protein SA, splice acceptor, LTR, long terminal repeat; SH; SH3 domain binding sequence (column 4, figure 2). Thus, Soriano taught a method of targeting region that is flanked on each side by a homology clamp, such that a double-crossover recombination between each of the homology clamps and their corresponding endogenous gene sequences result in replacement of the portion of the endogenous gene locus by the targeting region. However Soriano et al do not teach a method of using shRNA construct in rosa26 locus.

It would have been obvious for one of ordinary skill in the art at the time of invention to modify the method the construct disclosed by Beach to include the shRNA construct into a specific locus by homologous recombination in murine ES cells to

generate mice having a single-copy of a transgene inserted at a chosen site in the genome. Bronson provided motivation by emphasizing that the use of a chosen site for a single copy of a transgene avoids many of the problems associated with randomly inserted transgenes (see page 9072, col. 1, last paragraph). Furthermore, Soriano had already disclosed the methods and vector constructs for the production of non-human transgenic animals, which ubiquitously express a heterologous DNA segment in Rosa 26 locus. The skilled artisan would have been motivated to make transgenic nonhuman animal that comprises stably integrated expression vector comprising an shRNA into a specific locus such as ROSA26 or HPRT by homologous recombination as discussed by Bronson and Soriano, as it would have suppressed the expression of transgene for sustained period.

One who would practiced the invention would have had reasonable expectation of success because Beach et al had already described a method for gene knockdown in a mice by transiently as well as stably expressing shRNA construct and it would have only required routine experimentation that were disclosed by Bronson and Soriano before filing of this application. One of ordinary skill in the art would have been motivated to combine the teaching of Beach, Bronson and Soriano because a method of gene knockdown in a mouse comprising a shRNA construct under control of a ubiquitous promoter into a specific ROSA26/HPRT locus would have provided stable and sustained inhibition of transgene.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Claims 1-3, 5-24, 26-27 and 29-30 rejected under 35 U.S.C. 103(a) as being unpatentable over Beach et al. (US patent Publication no. 2003/0084471, dated 5/1/2003, effective filing date 1/22/2002); Bronson et al (Proc Natl Acad Sci U S A 1996; 93:9067–9072); Soriano et al (US patent 6,461,864, October 8, 2002) and Ohkawa et al (Hum Gene Ther. 2000; 11 (4): 577-85; IDS).

The combined teachings of Beach, Bronson and Soriano have been discussed above and are relied upon in same manner.

Ohkawa et al teach several constructs composed of the human U6 snRNA promoter and sequences derived from the gene for the tetracycline operator of a prokaryotic tetracycline resistance transposon (abstract). Ohkawa also disclose that expression of the promoter of the human gene for U6 snRNA that contains tet O sequences between the PSE (Figure. 1 and 2) and a TATA box could be be efficiently repressed in cells with the Tet repressor and that this repression can be reversed by tetracycline. Ohkawa et al used this expression system to control the function of an antisense RNA for a fusion gene composed of genes for epidermal growth factor receptor (EGFR) and green fluorescent protein (GFP) and expression of this chimeric gene could be efficiently and rapidly inhibited by tetracycline. However Ohkawa et al do not teach a method to gene knockdown in a nonhuman vertebrate.

It would have been obvious for one of ordinary skill in the art at the time of invention to modify the construct and method disclosed by Beach to include inducible promoters for shRNA construct wherein operator sequence consist tet as disclosed by Ohkawa. The skilled artisan would be further motivated to include this construct in a specific locus by homologous recombination in murine ES cells to generate mice having a single-copy of a transgene inserted at a chosen site in the genome wherein transgene could be regulated by tetracyline. Ohkawa provided the provided motivation by showing that tet based system could control the expression of transgene, while Bronson emphasized the use of a chosen site for a single copy of a transgene avoids many of the problems associated with randomly inserted transgenes (see page 9072, col. 1, last paragraph). Furthermore, Soriano had already disclosed the methods and vector constructs for the production of non-human transgenic animals, which ubiquitously express a heterologous DNA segment in Rosa 26 locus. The skilled artisan would have been motivated to make transgenic nonhuman animal that comprises stably integrated expression vector comprising an shRNA into a specific locus such as ROSA26 or HPRT

by homologous recombination as discussed by Bronson and Soriano as it would have suppressed the expression of transgene for sustained period.

One who would practiced the invention would have had reasonable expectation of success because Beach et al had already described a method for gene knockdown in a mice by transiently or stably expressing shRNA construct and it would have only required routine experimentation that were disclosed by Ohkawa, Bronson and Soriano, before filing of this application to a method for gene knockdown in a nonhuman vertebrate as recited in the instant application. One of ordinary skill in art would have been motivated to combine the teaching of Beach, Bronson, Ohkawa and Soriano because a method of gene knockdown in a mouse comprising a shRNA construct under control of a tet based inducible promoter into a specific ROSA26/HPRT locus would have provided stable and sustained regulated inhibition of transgene.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Conclusion

No Claims allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Anoop Singh whose telephone number is (571) 272-3306. The examiner can normally be reached on 9:00AM-5:30PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on (571) 272- 0735. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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